Environmental Health Perspectives Supplements
Vol. 101 (Suppl. 3): 43–46 (1993)

Dip-Strip Method for Monitoring Environmental Contamination of Aflatoxin in Food and Feed: Use of a Portable Aflatoxin Detection Kit

by R. B. Sashidhar

Aflatoxin contamination of food and feed have gained global significance due to its deleterious effect on human and animal health and its importance in the international trade. The potential of aflatoxin as a carcinogen, mutagen, teratogen, and immunosuppressive agent is well documented. The problem of aflatoxin contamination of food and feed has led to the enactment of various legislation. However, meaningful strategies for implementation of this legislation is limited by nonavailability of simple, cost-effective method for screening and detection of aflatoxin under field conditions. Keeping in mind the analytical constraints in developing countries, a simple-to-operate, rapid, reliable, and cost-effective portable aflatoxin detection kit has been developed. The important components of the kit include a hand-held UV lamp (365 nm, 4 W output), a solvent blender (12,000 rpm) for toxin extraction, and adsorbent-coated dip-strips (polyester film) for detecting and quantifying aflatoxin. Analysis of variance indicates that there were no significant differences between various batches of dip-strips ($p > 0.05$). The minimum detection limit for aflatoxin B_1 was 10 ppb per spot. The kit may find wide application as a research tool in public health laboratories, environmental monitoring agencies, and in the poultry industry.

Introduction

Aflatoxins are potent carcinogenic, mutagenic, teratogenic, and immunosuppressive agents produced as secondary metabolites by the fungal species *Aspergillus flavus* and *A. parasiticus* (*1*). Aflatoxin contamination of food and feed have gained global significance due to its deleterious effect on human and animal health and its importance in international trade (*2–4*). In view of the hazardous nature of aflatoxin, regulatory measures on its content in food ranging from 0.50 ppb have been introduced in various countries all over the world (*5*). The government of India has fixed the tolerance limit of 30 ppb for agricultural commodities under the Prevention of Food Adulteration act (*6*). However, in India and in many developing countries of the world, meaningful strategies for implementing legislation is limited by nonavailability of simple, rapid, cost-effective, and reliable analytical methods for screening and detecting aflatoxins, which could be easily performed either in the laboratory or under field conditions.

Department of Biochemistry, Osmania University, Hyderabad-500 007, India.

Various analytical techniques such as TLC, HPLC, HPTLC, radioimmunoassay, and ELISA have been developed for quantifying aflatoxins in food, feed, and biological fluids (*7,8*). Most of these methods require skilled manpower, sophisticated and expensive equipment, and elaborate laboratory setup for analysis and thus are not suitable for routine monitoring and screening of aflatoxins in agricultural commodities In the recent past, simpler techniques such as minicolumn chromatography and pressure minicolumn techniques have been developed and collaboratively evaluated (*7,9,10*). However, these minicolumn methods require regular laboratory facilities and a regular supply of prepacked columns, and the main drawback is that different aflatoxins cannot be resolved by this method. This paper describes the development of a portable aflatoxin detection kit (Aflakit) for screening and detecting aflatoxin under field conditions.

Materials and Methods

All reagents and solvents used were of analytical grade. Aflatoxin B_1 and B_2 standards were procured from Sigma Chemical Company (St. Louis, MO).

The components of the Aflakit are *a*) a hand-held longwave UV lamp, *b*) a portable sample grinder, *c*) a hand-held

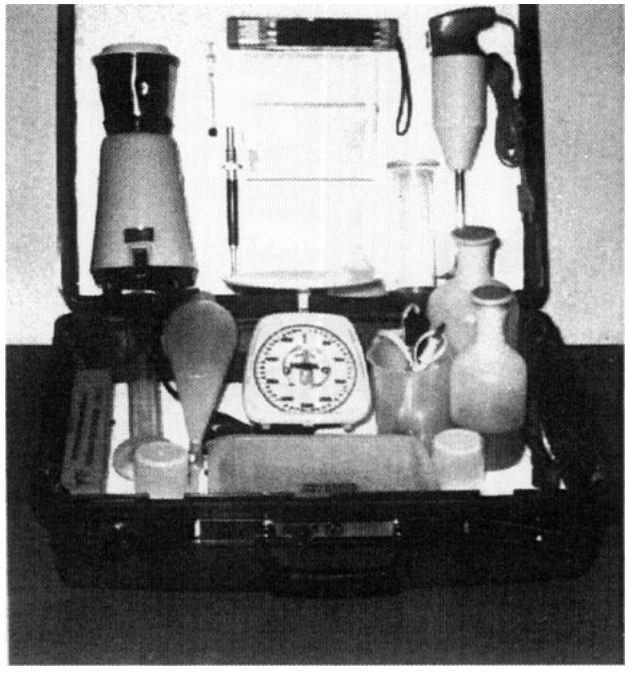

FIGURE 1. The Aflakit.

solvent blender, *d*) a balance, *e*) dip-strips, *f*) a microsyringe and dispenser, *g*) a separating funnel filtration heating coil and cleanup unit, *h*) solvents and reagents, and *i*) a mini-developing chamber. All these components were housed in a fiber-glass container (Fig. 1).

The hand-held UV lamp was designed and developed as a part of the kit. The UV lamp has 4 W output of longwave (356–370 nm) UV light. The lamp intensity is capable of illuminating 10 ng/spot of aflatoxin B_1 on a TLC plate at a distance of 10 cm. The portable sample grinder is suitable for grinding 50 g of sample. The hand-held solvent blender operates at 12,000 rpm and is suitable for rapid extraction of aflatoxin from a powdered sample. The dip-strips are made of polyester film (20 × 20 cm; 200 μm thick) and are coated with 250-μm thick silica gel (TLC grade) with soluble starch as binder and activated at 110°C for 1 hr. After activation, strips were cut to 40 mm × 120 mm long and used as dip-strips for aflatoxin analysis.

Method

Agricultural commodities were classified as low- and high-fat samples. For low-fat samples (e.g., maize, sorghum); 100 g of representative sample was ground to a fine powder with the portable grinder. Fifty grams of the homogenized sample was taken into a 500-mL beaker (polypropylene), along with 250 mL methanol:water (55:45 water containing 8% NaCl). The sample was blended with the hand-held blender for 3 min at 12,000 rpm. The sample was later filtered, and 50 mL of the filtrate was taken into separating funnel, along with 100 mL hexane followed by 50 mL of petroleum ether to defat the sample. The aqueous phase was separated, and to this 25 mL of reagent 1 (98.5 mL water, 1.5 mL glacial acetic acid, 20% zinc acetate, 0.5% EDTA, and 0.5% cupric carbonate) was added and thoroughly shaken. Aflatoxin was later extracted into 20 mL of chloroform. The chloroform layer collected was passed through a cleanup column containing neutral alumina (500 mg) and anhydrous sodium sulfate (2 g). The chloroform layer was evaporated to dryness, and the extracted toxin was redissolved in benzene:acetonitrile (98:2, 50–250 μL). Five microliters of aflatoxin extract was spotted onto a dip-strip and developed in the chloroform:acetone (95:5) solvent system for 12–15 min in a mini-developing chamber. The aflatoxins in the sample were visualized under the hand-held UV lamp.

For high-fat commodities (groundnut, cottonseed, sunflower seed), samples were first defatted. A 50-g sample was blended with 100 mL hexane and 100 mL petroleum ether for 3 min in the presence of a celite-540 filter aid. To the defatted sample, 250 mL of methanol:water (55:45) extraction solvent was added and blended for 3 min at 12,000 rpm. The rest of the procedure was similar to that of low-fat sample processing (Fig. 2). After the detection of aflatoxin on the dip-strip, the toxin was quantitated fluoro-densitometrically on the Bio-Med Laser scanning ID densitometer (*7*).

For aflatoxin B_1 spiking, aflatoxin-free samples of maize, sorghum (white variety), groundnut, cottonseed, and sunflower seed were spiked with pure aflatoxin B_1 at

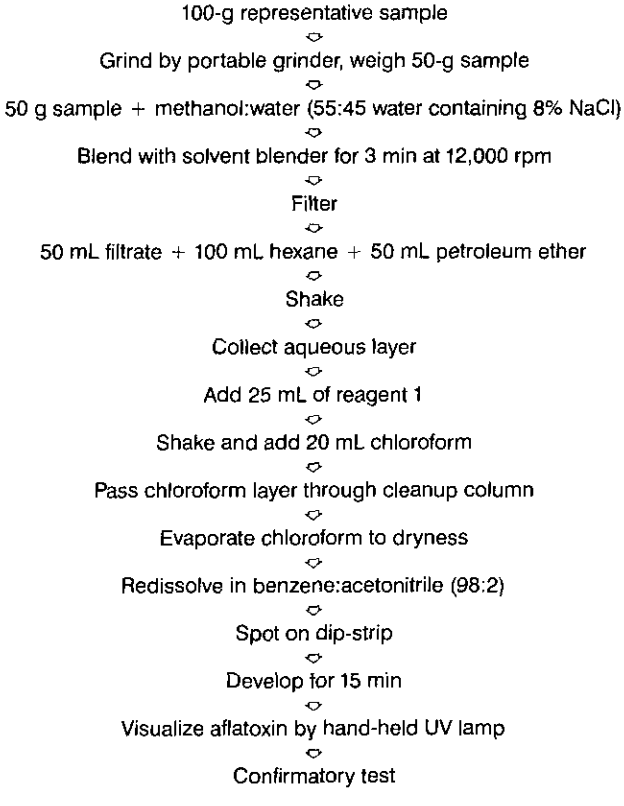

FIGURE 2. Schmatic representation of extraction and detection of aflatoxin by the Aflakit.

30 ppb (30 μg/kg) and 100 ppb (100 μg/kg). These spiked samples were used for recovery studies to assess the extraction method developed.

Results and Discussion

The Aflakit was developed to meet the need of field application. The methods and use of the Aflakit were aimed at rapid screening and detection of total aflatoxins in contaminated samples. Aflakit includes a sample processing unit and a portable hand-held longwave UV lamp for aflatoxin detection. The kit offers a simple, cost-effective, and reliable method for monitoring aflatoxin in food and feed. The polyester dip-strips afford easy handling, unlike regular TLC plates. Tables 1 and 2 show the variation and results of the analysis of variance (ANOVA) for the dip-strips used for aflatoxin detection. There was no significant difference between the batches of dip-strips made, and no variation was observed between the strips within a batch ($p > 0.05$). The separated aflatoxins were visualized under the hand-held UV lamp. The Rf value for aflatoxin B_1 and B_2 were observed to be 0.21 and 0.18, respectively. The time taken per sample analysis was less than 2 hr. Further chemical confirmatory tests based on trifluoroacetic acid can be used to confirm the identity of aflatoxin B_1. Table 3 gives the recovery of aflatoxin B_1 spiked at 30 ppb and 100 ppb (μg/kg) in various agricultural commodities. The recoveries ranged from 79 to 89% and from 87 to 96% in samples spiked with aflatoxin B_1 at 30 ppb and 100 ppb, respectively. The coefficient of variation at the 30 ppb level of aflatoxin spiking ranged from 1.5% to 7% and for 100 ppb ranged from 1.0 to 4.2%. Various market samples were analyzed and screened for aflatoxins using the Aflakit. Table 4 gives the level of aflatoxin contamination in these samples.

Aflatoxin detection by Aflakit is based on the well-established physicochemical method of thin-layer chromatography. The major advantage of the Aflakit is its ease of handling and the ability to analyze food and feed samples under field conditions. After screening the samples for aflatoxins on dip-strips, they can be brought back or mailed to the central reference laboratory for quan-

Table 1. Interbatch and intrastrip variation in dip-strips.

	Area under aflatoxin B_1 peak (integrator units)[a]			
	Strip no.			
Batch no.	1	2	3	4
1	14.40	14.39	14.42	14.37
2	13.90	14.40	14.50	13.95
3	14.79	14.57	14.87	14.46
4	14.81	14.93	14.49	14.79
5	14.30	14.37	14.13	14.42
Mean	14.44	14.53	14.48	14.37
SD	0.375	0.236	0.263	0.305
CV	2.6	1.6	1.8	2.1

CV, coefficient of variation.
[a]Concentration of aflatoxin B_1 = 20 ppb (20 μg/kg).

Table 2. Analysis of variance.

Source	df	Sum of squares	Mean squared	*F* ratio
Strips	3	0.0657	0.0219	0.534*
Batches	4	0.0894	0.0223	0.543*
Error	12	0.4928	0.0410	
Total	19	0.6479		

df, degrees of freedom.
[a]Concentration of aflatoxin B_1 = 20 ppb (20 μg/kg).
*$p > 0.05$.

Table 3. Recoveries of spiked aflatoxin B_1 in various agricultural commodities.[a]

Level of aflatoxin B_1 spiked, μg/kg	Commodity, μg/kg				
	Maize	Sorghum	Groundnut	Cottonseed	Sunflower seed
30					
Mean	26.8	27.28	25.2	23.92	24.11
SD	0.42	0.926	0.565	1.627	1.229
100					
Mean	96	95.17	91.65	87.6	89.15
SD	2.262	2.380	0.919	3.394	3.782

[a]Values are means of triplicate determination.

Table 4. Analysis of aflatoxin in market samples by the Aflakit.

	Visual detection[a]		Fluorodensitometric detection, μg/kg[b]	
Commodity	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin B_1	Aflatoxin B_2
Peanut butter	+	+	40.2	–
Groundnut	+	–	108.6	
Defatted groundnut flour	+	+	220.5	70.4
Groundnut cake	+	+	140.3	42.2
Maize (popcorn)	+	–	90.0	–
Copra		– –	–	–
Cottonseed	+	+	120.7	32.4
Sunflower seed	–	–	–	–
Poultry feed	+	+	65.8	–

[a](+) sample positive for aflatoxin, (–) sample negative for aflatoxin.
[b]Values are means of replicate observations.

tification by fluorodensitometry. Recently, commercial ELISA kits for aflatoxin detection have been marketed in developed countries. These kits are based on antigen-antibody reactions. However, these kits have a very limited shelf-life and are highly sensitive to temperature during transportation, especially in tropical countries like India. Further, the ELISA kits are limited by their nondetection of other aflatoxins, which co-occur along with aflatoxin B_1 in contaminated samples. The Aflakit has an additional advantage of detecting total aflatoxin content in the contaminated sample.

The Aflakit is potentially a viable analytical tool for routine surveillance of aflatoxin contamination in food and feed and successful implementation of the Prevention of Food Adulteration Act. Further, Aflakit may find wide application as a tool of operational research in public health laboratories, veterinary health organizations, the poultry industry, and environmental monitoring agencies.

This work has been funded and supported by the Council of Scientific and Industrial Research (CSIR), New Delhi (no. 9/329/91 EMB II). The author thanks the University Grant Commission, New Delhi, for providing instrumentation facilities under the COSIST programme at the department. Thanks are also due to H. V. V. Murthy of the National Institute of Nutrition, Hyderabad, for useful discussions.

REFERENCES

1. WHO. Mycotoxins. Environmental Health Criteria 11, World Health Organization, Geneva, 1979.
2. Mycotoxins 1987. Report on the Second Joint FAO/WHO/UNEP. International Conference on Mycotoxins, Bangkok, Thailand. Food and Agriculture Organization, Rome, 1988.
3. Aflatoxin – Research Analysis, Control. JAOCS 66: 1398–1413 (1989).
4. Ellis, W. O., Smith, J. P., and Simpson, B. K. Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and method of control. CRC Crit. Rev. Food Sci. Nutr. 30: 403–439 (1991).
5. van Egmond, H. P. Current situation on regulation for mycotoxins: overview of tolerance on status of standard methods of sampling and analysis. Food Add. Contamin. 6: 139–88 (1988).
6. Trivede, Ed. A. P. Mathur's Commentries on Prevention of Food Adulteration Act (PFA), 1954, India, 8th ed. Eastern Book Company, Lucknow, India, 1986.
7. Egan, N., Ed. Environmental Carcinogens: Selected Methods of Analysis, Vol. 5, IARC Publication No. 44. International Agency for Research on Cancer, Lyon, 1982.
8. Peska, J. J. Enhanced surveillance of food borne mycotoxins by immunochemical assays. J. Assoc. Off. Anal. Chem. 71: 1075–1081 (1988).
9. Sashidhar, R. B., Ramakrishna, Y., and Bhat, R. V. Rapid detection of aflatoxins by pressure minicolumn technique. Anal. Lett. 21: 507–518 (1988).
10. Sashidhar, R. B., Bhat, R. V., and Vasanthi, S. Rapid detection of aflatoxins by pressure minicolumn technique: a collaborative trial. Curr. Sci. 58: 882–885 (1989).